

## Rapid Assessment Methodology for the Detection of Microbiological Indicators

To assess beach water quality, the Department of Environmental Services (NHDES) monitors fecal indicator bacteria levels at coastal beaches on a routine basis.

Unfortunately, sample analysis can take anywhere from 24 to 48 hours. Because it takes at least 24 hours to receive results, beach managers and the public are not informed of water quality problems until after the public may have been exposed. This is an issue facing beach officials throughout the world, and is a top



priority of the US Environmental Protection Agency (EPA). The EPA, universities and private entities are researching rapid assessment methods to enumerate bacteria and viruses. These methods will allow beach officials to post advisories on the same day water quality is impaired, thus, better protecting public health. There are three different rapid assessment method technologies available: Molecular surface recognition, nucleic acid detection and enzyme/substrate based methods. All rapid assessment methods will take less than two hours to obtain results.

Molecular surface recognition methods capture and/or label the target bacterium by binding to molecular structures on the exterior surface or in its genetic material.

Analyses of coastal beach water samples currently employ culture-based methods for the detection of Enterococci bacteria, an indicator for fecal pollution in marine water. The quickest culture-based method takes up to 24 hours to provide results. Now, a new method is being developed to enumerate Enterococci. This new method uses Transcription-Mediated Amplification (TMA) with a fluorescently-labeled probe to amplify a specific region of Enterococci ribosomal RNA (rRNA).

The TMA rapid assessment method is currently being tested in Southern California. Development of methods is moving quickly and will likely come to execution within five years. One reason why the new technology is not currently being used is that it is more expensive. Once this procedure is widely and routinely used, the expenses will lower. This rapid assessment method is very beneficial because it will allow beach managers to take action towards protecting the public from exposure to waterborne pathogens on the same day that the water is sampled.

Another rapid assessment method that is being developed for the detection of fecal indicators is called Quantitative Polymerase Chain Reaction (QPCR). QPCR is a nucleic acid detection method that targets genetic material of bacteria, viruses or protozoan indicators. QPCR is being used to test for both *E. coli* and Enterococci. Results while

using this method can be obtained on an average of two hours after sampled. These collections have demonstrated 85-90 percent agreement with existing routine methods. This method can be used to detect other water quality indicators such as *Bacteroides thetaiotamicron* and human enterovirus. Studies indicate that ratios of *B. thetaiotamicron* may provide useful information as to the source of fecal contamination in samples collected.

The final rapid assessment technology methods available are the enzyme/substrate based methods. These methods pair chromogenic or fluorogenic substrate methods already widely used with advanced optical or electrical detectors. These methods are directed at reducing the incubation periods of current membrane filtration methods. Some of these methods measure excitation and absorbance of the fluorescent metabolite of Enterococci using a fluorometer which will speed up the rate of detection. A popular type of enzyme/substrate method is called the Dual-Wavelength Fluorimetry (DWF).

These rapid assessments methods are currently being tested for its accuracy, sensitivity and efficiency. Research indicates that these new methods will be made available within the next five years. Once these technologies are made available and laboratories adopt the methods, beach management will be able to better protect public health. With assistance from EPA Beach Grants, New Hampshire is hoping to employ these methods once they become available.